

THE EFFECT OF SEX AND ALLATECTOMY ON THE OXYGEN  
CONSUMPTION OF THE THORACIC MUSCULATURE  
OF THE INSECT, *LEUCOPHAEA MADERAE*<sup>1</sup>

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Previous results of metabolic studies in a variety of insect species clearly illustrate the difficulty in making generalizations (Sacktor, Thomas, Moser and Block, 1953). Thus it is not too surprising that, in contrast to the work of Barron and Tahmisian (1948) in *Periplaneta americana*, no sex difference was found in the present study regarding the endogenous oxygen consumption of the musculature of *Leucophaea maderae*. A further, more interesting result is that the thoracic muscles of both sexes of *Leucophaea maderae* increase their oxygen consumption after allatectomy. This finding is in seeming contrast to that of Thomsen (1949) who observed a decrease in the oxygen consumption of the whole intact fly, *Calliphora erythrocephala*, following allatectomy. However, this discrepancy may perhaps be explained on the basis of the work of L'Hélias (1953) who found an increase in the carbohydrate content of tissues and a decrease in blood sugar of *Dixipus morosus* after the removal of the corpora allata (see Discussion).

Preliminary tracer experiments were undertaken in order to ascertain whether there was any correlation, *in vivo*, between the increased oxygen consumption and phosphorylation, but they were terminated before any definite conclusions could be drawn.

METHODS

The determination of oxygen consumption ( $\text{O}_2$ ) was carried out at 26° C. in a conventional Warburg apparatus, each vessel containing a total volume (including muscle and KOH) of 3 ml. of Bělař's solution (.9% NaCl; .02% KCl; .02%  $\text{CaCl}_2$ ) buffered at pH 6.8 with .01 M phosphate. The thoracic muscles, weighing approximately 150 mg., of a single animal were kept on ice (1–3° C.) in Bělař's solution, without glucose or other substrate, weighed, separated with the aid of needle probes into very small pieces, and placed in the Warburg vessels. Oxygen consumption measurements were initiated, after a 15-minute equilibration period, in less than an hour after the muscles were dissected out. The moisture content, determined on normal animals of both sexes and allatectomized animals at the end of a few of the Warburg runs, averaged 80%. Allatectomy was performed soon after emergence and the operated animals were used two to three months afterwards. The controls were always of the same adult age.

The preliminary tracer experiments were carried out *in vivo*. Two microcuries

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of a high activity solution of neutralized ortho sodium phosphate <sup>32</sup> were injected into the body cavity of each of the animals. They were sacrificed 24 hours later, the thoracic muscles and the gut were dissected out, rinsed in several changes of phosphate-free Bělař's solution, blotted, weighed, and frozen on dry ice, prior to homogenizing in iced 10% trichloroacetic acid. The phosphorus was fractionated by the method described by Schneider (1945).

Counting was carried out with a thin window (1.4 mg.) G-M tube on aliquots of the ashed fractions, containing fairly uniform amounts of phosphate.

### RESULTS

Table I shows that there was no significant difference between the sexes in the  $\text{QO}_2$ 's of the thoracic muscles, 11 males averaging  $3.25 \pm .28$  and 10 females averaging  $3.35 \pm .28$  cubic mm./mgm. dry wt./hour. Deviation is expressed as standard error. Inasmuch as the oxygen uptake of the muscles was linear for about

TABLE I  
*Oxygen consumption of roach muscle Leucophaea maderae*  
Male versus Female

Experiment No.	Males	$\text{QO}_2$	Females
1	2.7		2.9
2	2.5		2.1
3	3.0		2.5
4	2.8		3.9
5	1.7		4.6
6	4.7		3.3
7	3.9		2.3
8	4.1		4.1
9	4.0		3.1
10	4.2		4.7
11	2.2		
Average	$3.25 \pm .28$		$3.35 \pm .28$

50 minutes, the  $\text{QO}_2$  values were calculated on the basis of the average oxygen consumption at 30 minutes. In a few trial experiments, we were able to confirm the results of Barron and Talmisian (1948) on *Periplaneta americana*, in which they found a larger oxygen consumption in male thoracic muscles than in females.

In another experimental series dealing with allatectomized as compared with normal control males and females, the muscles of 18 normal animals had a  $\text{QO}_2$  of  $3.50 \pm .22$  while 18 allatectomized males and females averaged  $4.26 \pm .20$ . These results are shown in Table II. The 20% difference is significant, as a "t" test gives a P of approximately .02.

The two series of experiments (male vs. female and normal vs. allatectomized) were done at widely separate times, and as slight advantage, statistical or otherwise, would be gained by pooling the two series of normal animals, the calculation was not made.

In preliminary, *in vivo*, tracer experiments, utilizing  $\text{P}^{32}$ , the 24-hour uptake into the acid-soluble (A.S.), phospholipid (P.L.), nucleic acid (N.A.) and residual phosphate (R.P.) fractions was determined. Eight animals, five normal and three

TABLE II  
*Oxygen consumption of roach muscle Leucophaea maderae*  
 Normal versus Allatectomized

Experiment No.	Normal	QO <sub>2</sub>	Allatectomized
1	4.3		4.8
2	2.5		3.7
3	3.9		4.5
4	4.6		5.8
5	3.3		4.2
6	2.3		3.8
7	4.1		4.4
8	3.1		4.2
9	4.7		5.9
10	3.0		4.1
11	2.8		3.8
12	1.7		2.8
13	4.7		5.2
14	3.9		4.4
15	4.1		4.0
16	4.0		3.3
17	4.2		4.8
18	2.2		3.0
Average	3.50 ± .22		4.26 ± .20

allatectomized, were used. Tissues were rinsed thoroughly to remove adhering tracer phosphorus. In terms of relative specific activities, that is, specific activity ratios of the insoluble fractions to the acid-soluble fraction, the ratios in the gut were two to five times higher than those of similar fractions of muscle. The higher relative specific activities of gut indicate that phosphate is metabolized at a higher rate in all fractions of gut than in those of muscle. This higher metabolic activity is supported by preliminary experiments in which the oxygen consumption of gut was also shown to be higher than that of muscle. However, as it was difficult to be certain of complete removal of normal gut bacteria, the reliability of the QO<sub>2</sub> values was questionable at that time.

TABLE III

*Uptake of radioactive phosphorus into muscle of normal and allatectomized Leucophaea maderae*

Date	Animal	Specific activity							
		Acid soluble	A.S./Blood	P.L.	P.L./A.S.	N.A.	N.A./A.S.	P.P.	P.P./A.S.
1-14-54	Normal	255	.66	12.4	.048	41.0	.160	37.4	.146
1-14-54	Normal	246	.64	10.8	.044	44.6	.182	39.0	.158
1-14-54	Allatectomized	377	—	14.0	.037	61.0	.161	82.8	.218
7-9-54	Normal	105	.44	5.0	.047	16.6	.158	14.0	.133
7-9-54	Normal	293	.45	11.6	.040	48.0	.164	51.6	.178
7-9-54	Normal	134	.54	7.2	.053	24.2	.181	24.4	.182
7-9-54	Allatectomized	154	.55	6.9	.045	29.9	.194	28.0	.181
7-9-54	Allatectomized	165	.42	8.1	.049	41.0	.248	33.9	.206

However, in the muscles, as shown in Table III, no significant differences could be found in the AS or PL fractions between normal and allatectomized roaches. For example, the relative specific activities of the phospholipids PL/AS of the normals average  $.046 \pm .004$  while those of the allatectomized average  $.044 \pm .005$ . Error is expressed here as standard deviation.

The specific activities of the N.A. and P.P. fractions appear closely related, which may mean that the two groups of compounds are metabolically related, that they have reached a similar equilibrium value, or that the method of separation is inadequate. However (although no statistical significance may be attached to the fact) the average of the relative specific activities of the allatectomized animals in the N.A. and the P.P. fractions, separately, is higher than that of the controls.

#### DISCUSSION

The increased oxygen consumption of the muscles of the allatectomized animals (Table II) indicates that the removal of the corpus allatum hormone may result in an elevation of the endogenous substrate concentration (glucose and glycogen) and/or the concentration of some oxidative enzymes in insect muscle. In allatectomized *Dixippus morosus*, L'Hélias (1953) found an elevated carbohydrate content in the tissues and a lowered blood sugar. If comparison of these two forms of insect is possible, one would, by analogy, expect a similar increase in the carbohydrate stores in the muscles of allatectomized *Leucophaea maderae*, which when removed from the *in vivo* situation and thrown upon its endogenous resources *in vitro*, would result in a higher oxygen consumption than that of the normal control muscles under the same circumstances. Furthermore, if the lowered blood sugar of *Dixippus morosus* holds for *Calliphora* as well, it would explain the results of Thomsen (1949) showing a lowered oxygen consumption of living allatectomized flies, for in the living animal the tissues might be constrained to the use of the lowered blood sugar and would consequently respire at a lower rate than controls with a normal blood sugar.

This hypothesis is, of course, tentative in view of the metabolic variation obtaining even in closely related species of insects.

The absence of a sex-correlated difference in muscle oxygen consumption shown here, although not obtaining in *Periplaneta americana* (Barron and Tahmisian, 1948) could have been predicted from the succinoxidase contents of muscles of males and females of these species, *Leucophaea maderae* and *Periplaneta americana*, as the results of McShan, Kramer and Schlegel (1954) which appeared while this work was in progress showed that there is no sex difference in the content of this oxidative enzyme in *Leucophaea maderae*, as compared to the three-fold difference found by Harvey and Beck (1953) in *Periplaneta americana*.

Little may be said concerning the results obtained with tracer phosphorus, as the few experiments completed show no changes comparable to the oxygen consumption data. However, it should be recognized that these values represent only gross phosphate fractions. Neither specific activity-time relations nor the correspondence between *in vivo* and *in vitro* P<sup>32</sup> uptake by this roach muscle was investigated.

Consequently the question of whether or not the increased oxygen consumption

of muscle of allatectomized *Leucophaea maderae* is coupled to phosphorylation, must remain the subject of further research.

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#### SUMMARY

1. Endogenous oxygen consumption of thoracic muscles of *Leucophaea maderae* was no higher in males than in females, but there was a significant increase in this parameter in allatectomized animals of both sexes.

2. Preliminary tracer phosphorus studies were made *in vivo* and the relative specific activities of the muscle fractions are shown.

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